MCAL Spectrophotometry

Instruments include:

- Cary 50 UV-vis Spectrophotometer
- Eclipse Spectrofluorometer
- HPLC Diode Array and Fluorescence
- ICP-OES with CCD detection

Spectrophotometry

- The instruments all have in common that they use the absorption or emission of light, in the form of electromagnetic radiation (EMR) as a means of measuring the presence and/or concentration of material in a solution.

Cary 50 and Eclipse

Cary 50 Spectrophotometer

- Mainly used to measures concentration of an analyte in solution

- Concentration based on absorption of light (incident minus final light beam) by analyte (Beer-Lambert law) at specific wavelength

- Need standards due to different extinction coefficients of different analytes.

Concentration

- Based on Beer's Lambert Law that concentration is proportional to the absorption of certain energy in the form of light

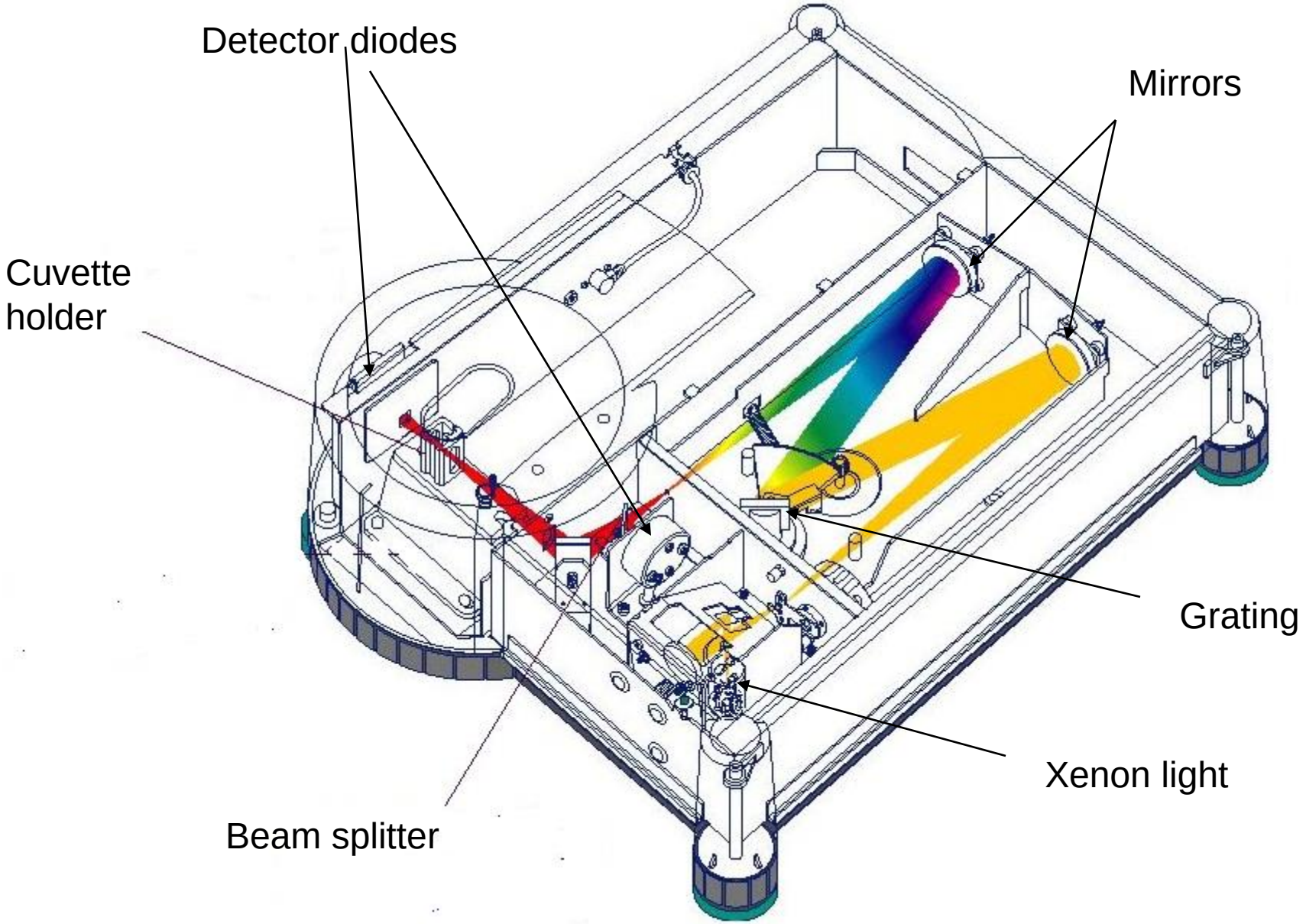
A(absorbance) = εcd

ε = extinction coefficient (constant for each compound)

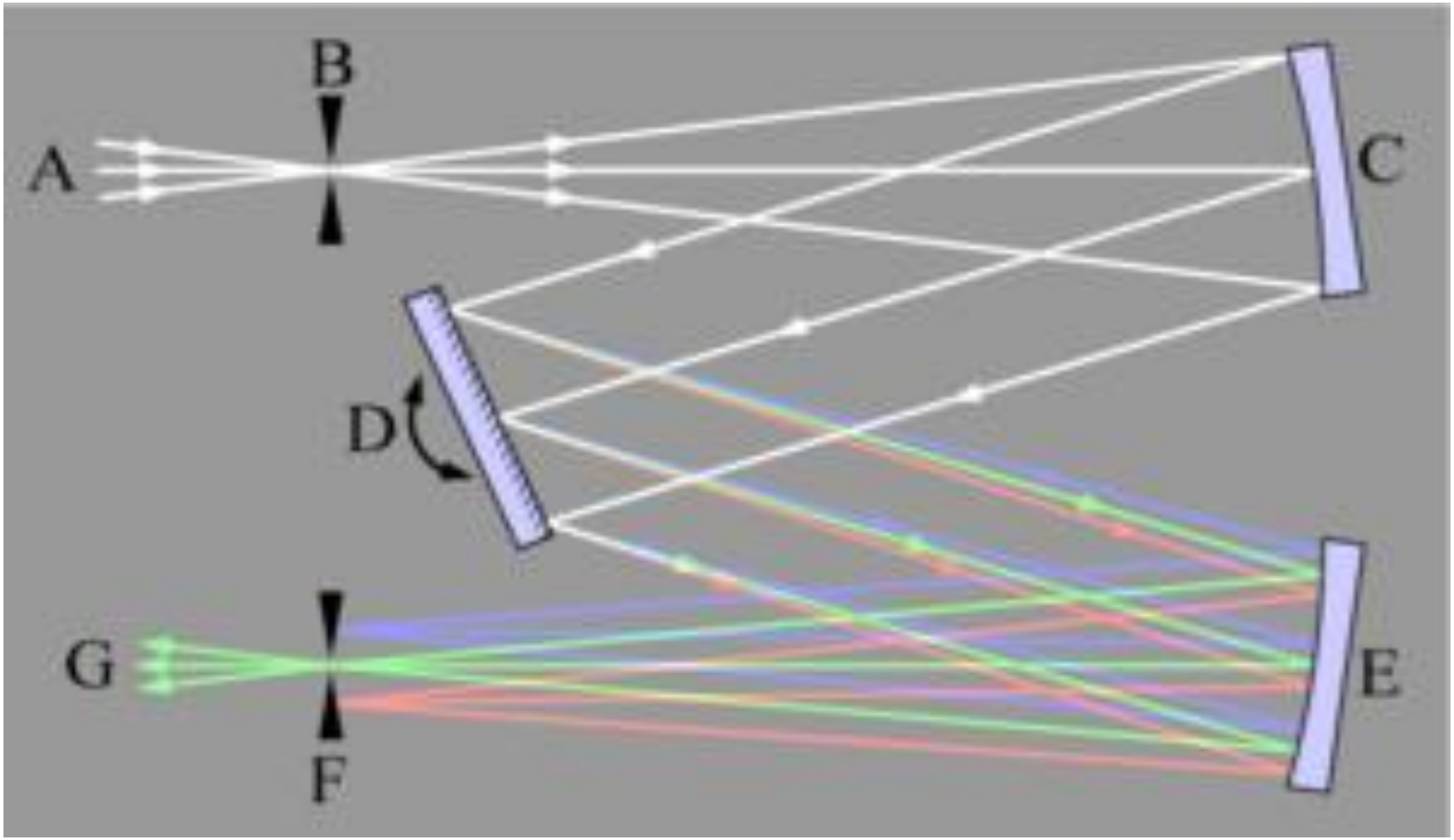
d = path length of cuvette =1cm

c = concentration of analyte

Cary 50 Uv-Vis spectrophotometer (measures absorption at specific wavelength)

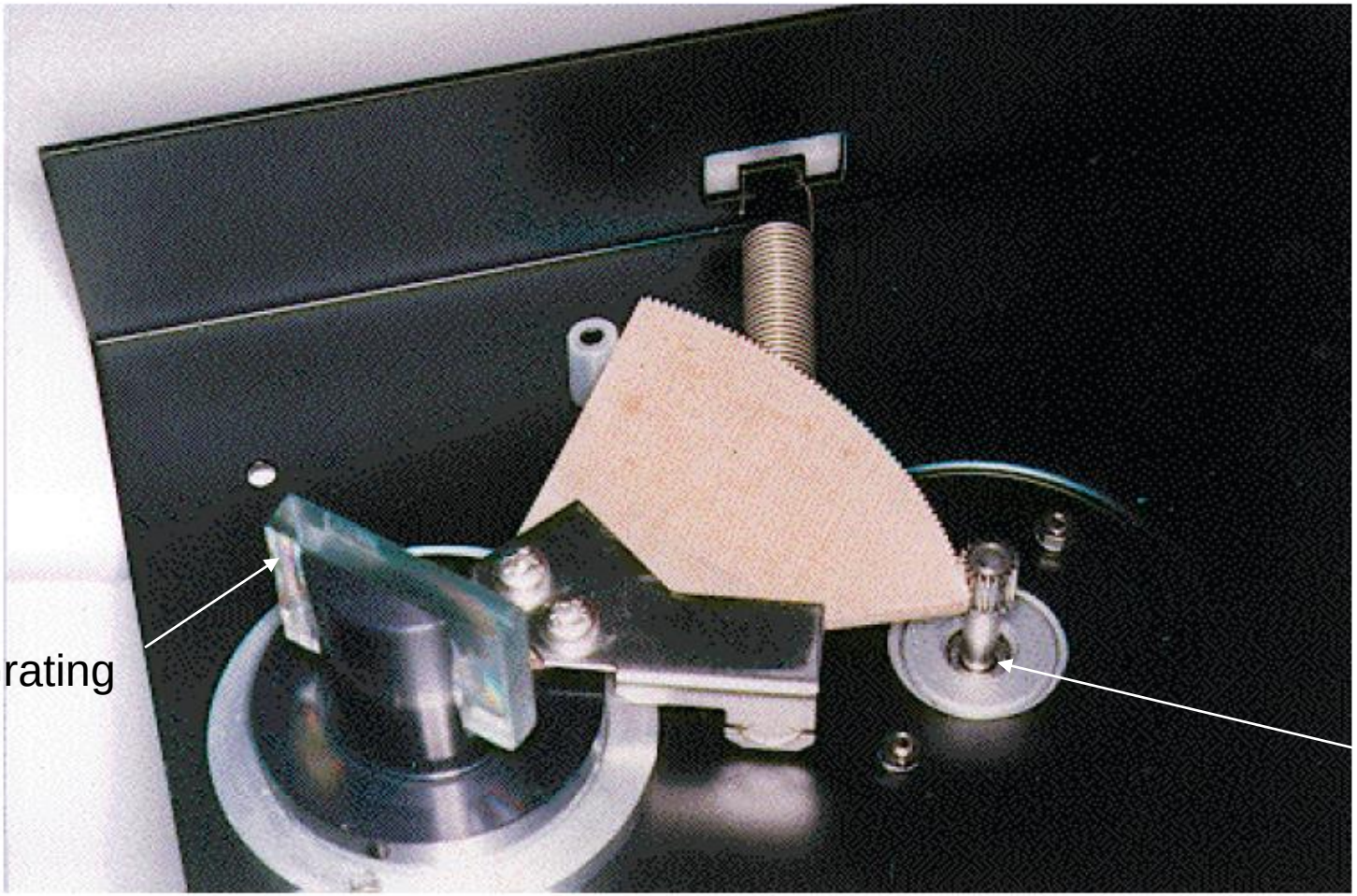


Czerny-Turner monochromator



In the common Czerny–Turner design,[1] the broad-band illumination source (**A**) is aimed at an entrance slit (**B**). The amount of light energy available for use depends on the intensity of the source in the space defined by the slit (width × height) and the acceptance angle of the optical system. The slit is placed at the effective focus of a curved mirror (the collimator, **C**) so that the light from the slit reflected from the mirror is collimated (focused at infinity). The collimated light is diffracted from the grating (**D**) and then is collected by another mirror (**E**), which refocuses the light, now dispersed, on the exit slit (**F**). In a prism monochromator, a reflective prism takes the place of the diffraction grating, in which case the light is refracted by the prism.
At the exit slit, the colors of the light are spread out (in the visible this shows the colors of the rainbow). Because each color arrives at a separate point in the exit-slit plane, there are a series of images of the entrance slit focused on the plane. Because the entrance slit is finite in width, parts of nearby images overlap. The light leaving the exit slit (**G**) contains the entire image of the entrance slit of the selected color plus parts of the entrance slit images of nearby colors. A rotation of the dispersing element causes the band of colors to move relative to the exit slit, so that the desired entrance slit image is centered on the exit slit. The range of colors leaving the exit slit is a function of the width of the slits. The entrance and exit slit widths are adjusted together.

Grating Scan



Varian Cary® 50 UV-Vis spectrophotometer

- Dual beam (split beam), Czerny-Turner **monochromator**, 190–1100 nm wavelength range, approximately 1.5 nm
- **Lamp** full spectrum Xe pulse lamp single source
- quartz overcoated **optics**, room light immunity, central control by PC with Windows® interface.
- **Grating** holographic, 27.5 x 35 mm, 1200 lines/mm, blaze angle 8.6° at 240 nm
- **Beam splitting system**
- **Detectors** 2 silicon diode detectors

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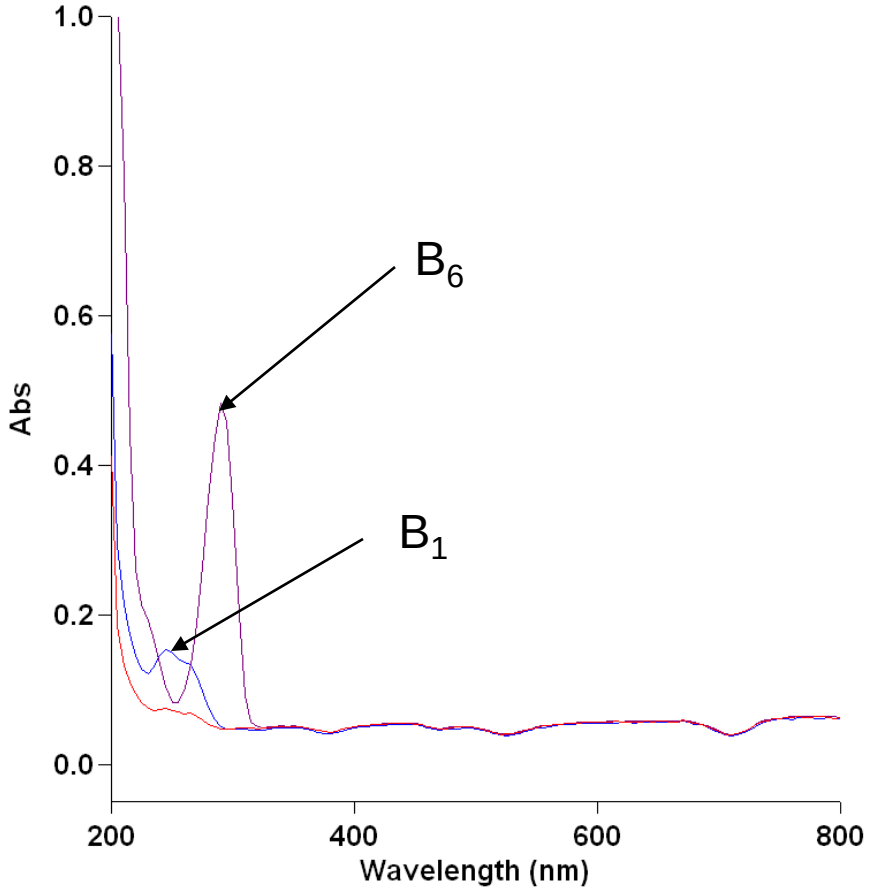
Xenon Flash Lamp Advantages

- The maximum scan rate is 24,000 nanometers (nm) per minute.
- Scan190–1100 nm in less than 3 seconds.
- Measure samples up to 3 Abs
- The Xenon lamp has a very long lifetime—3×10^9 flashes
- Room-light immunity

Uv-Vis Experiment

- The analysis involves solving a set of simultaneous equations which describe the absorbance behavior of a mixture of vitamins; B_1 (thiamine) and B_6 (pyridoxine) at different wavelengths.
- The number of equations, and likewise the number of wavelengths, equals the number of components in the mixture

Scan of Vitamin B_1 and B_6



Simultaneous Equations

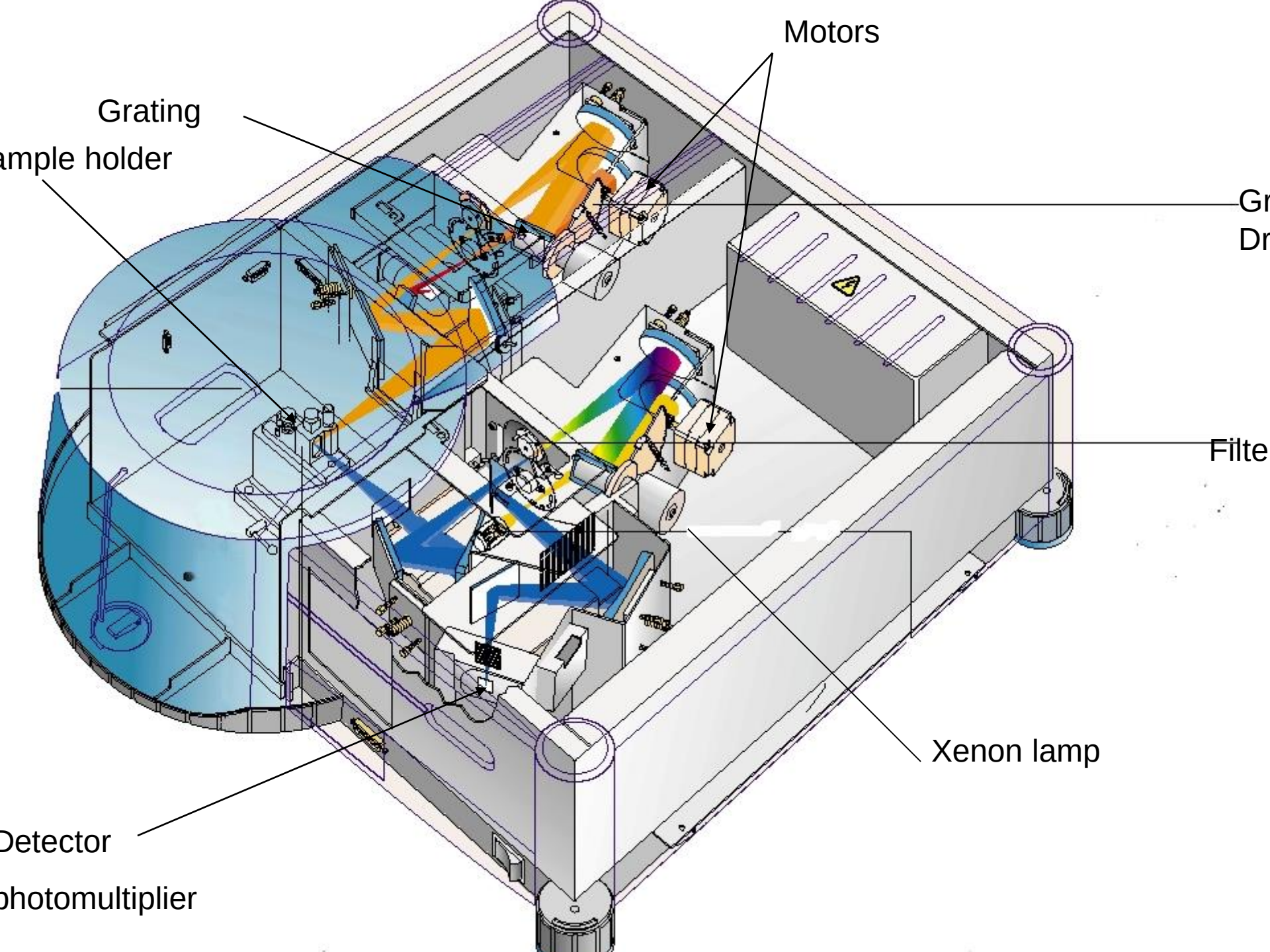
Mixture of B_1 and B_6:

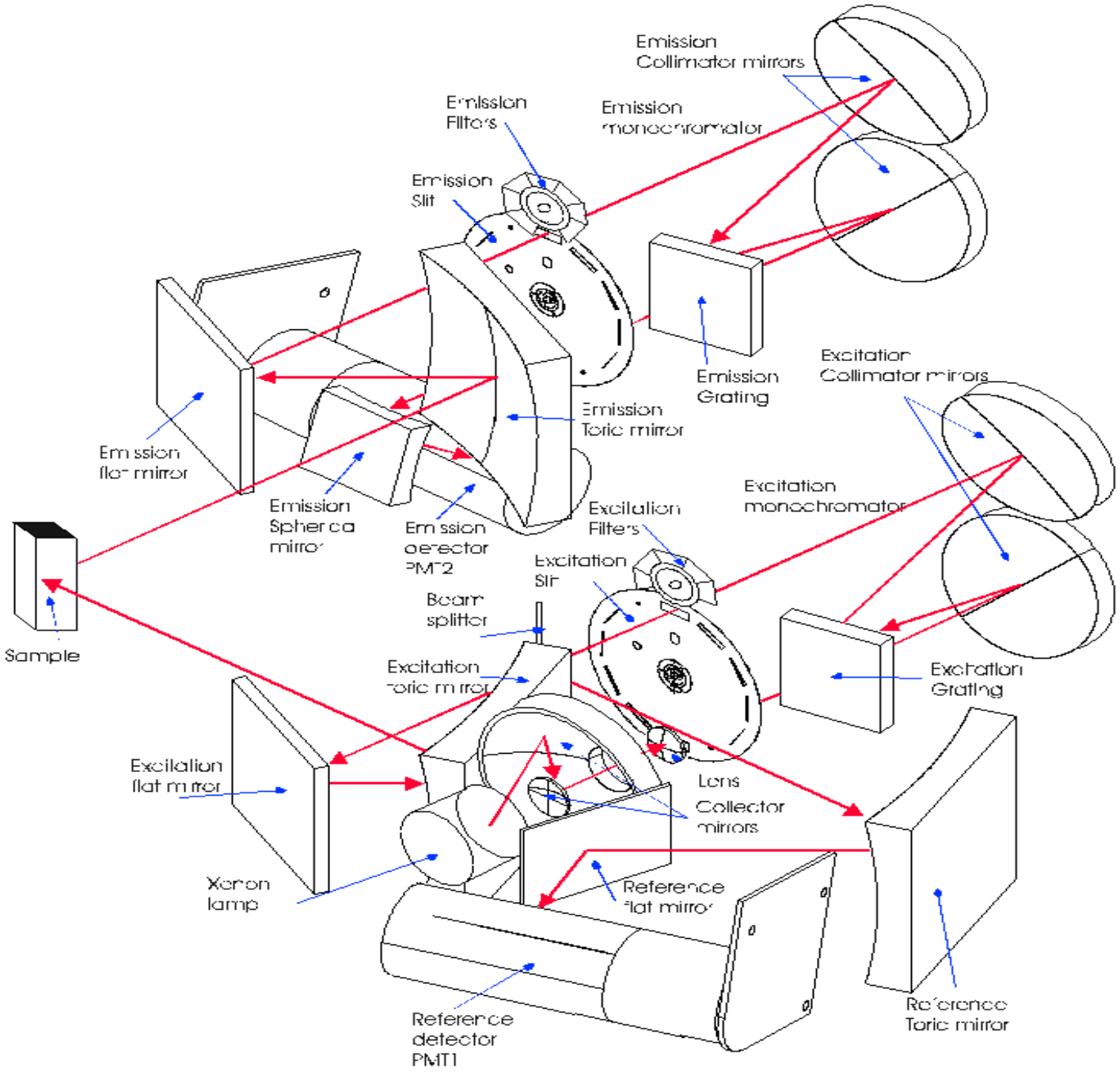
$$A_{245} = \varepsilon_{B1(245)}C_{B1} + \varepsilon_{B6(245)}C_{B6}$$

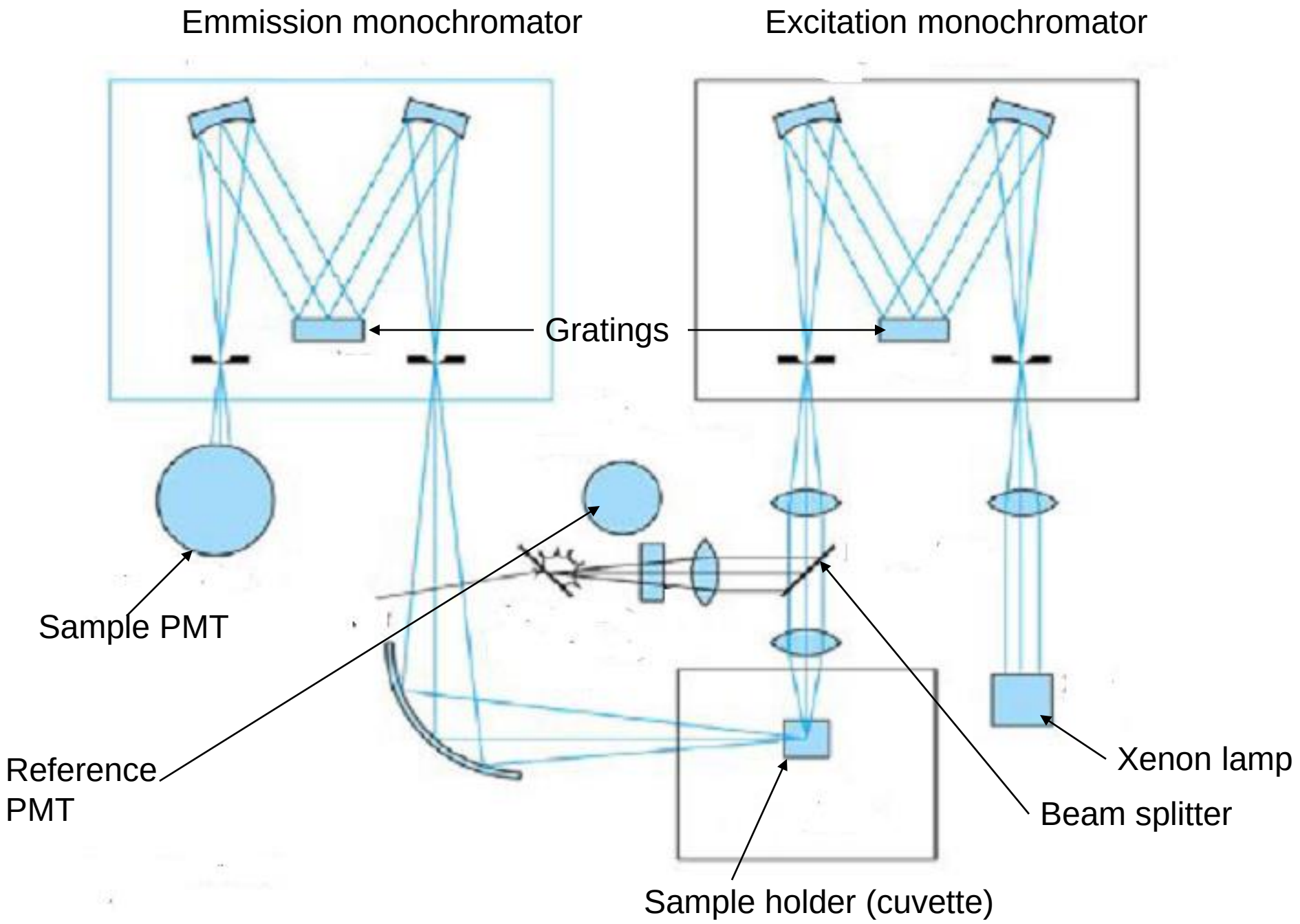
$$A_{290} = \varepsilon_{B6(290)}C_{B6} + \varepsilon_{B1(290)}C_{B1}$$

Spectrofluorometer

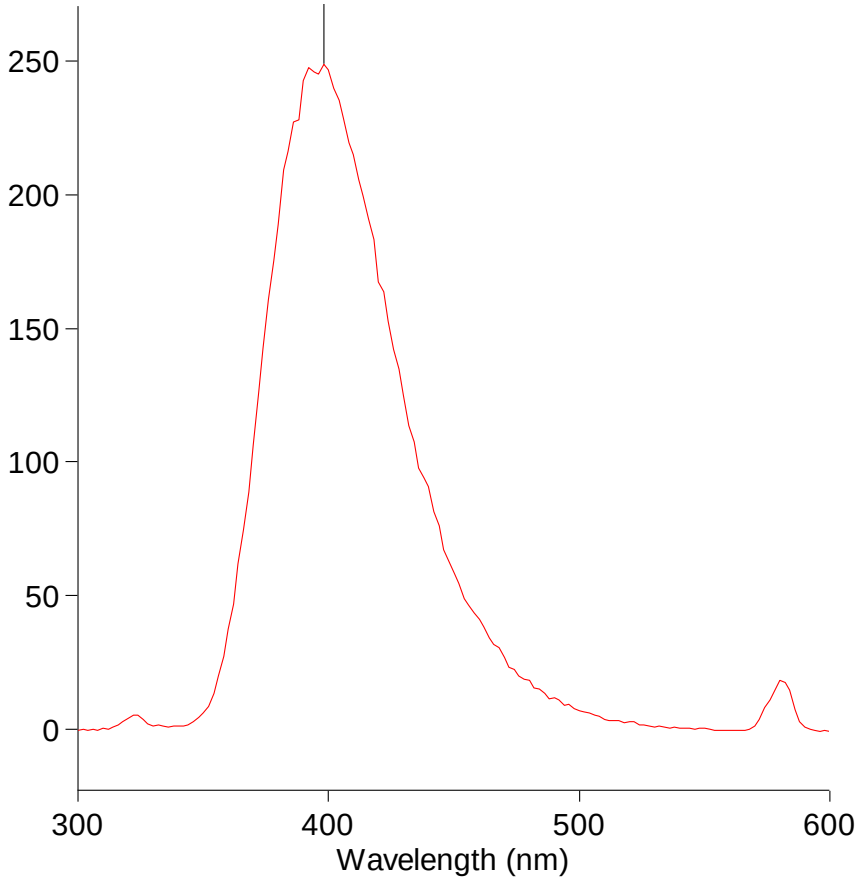
- Mainly used to measure the concentration of fluorescing compounds.
- Advantage - high sensitivity
- Disadvantage - low number of compounds fluoresce
- But - can make compounds fluoresce by binding a fluorescent compound


Motors
Grating
ample holder
Gr
Dr
Filte
Xenon lamp
Detector
photomultiplier


Emission
Collimator mirrors
Emission
Filters
Emission
monochromator
Emission
Slit
Emission
Grating
Excitation
Collimator mirrors
Emission
Toric mirror
Emission
flat mirror
Emission
Spherical
mirror
Emission
detector
PMT2
Excitation
monochromator
Excitation
Filters
Excitation
Slit
Beam
splitter
Sample
Excitation
Toric mirror
Excitation
Grating
Excitation
flat mirror
Lens
Collector
mirrors
Xenon
lamp
Reference
flat mirror
Reference
detector
PMT1
Reference
Toric mirror


Emmission monochromator
Excitation monochromator
Gratings
Sample PMT
Reference PMT
Xenon lamp
Beam splitter
Sample holder (cuvette)

Emmission Scan Eclipse Spectrofluorometer



HPLC with Diode Array Detector

Diode Array

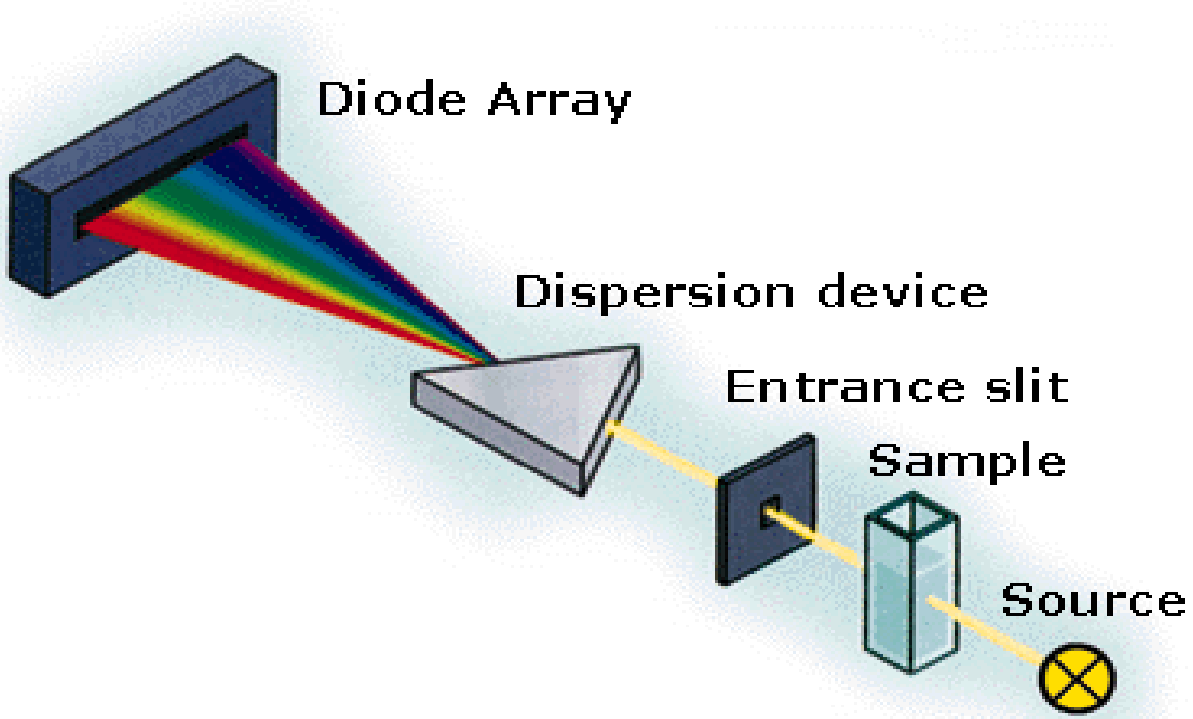
- The diode array uses a broad emission light source
- two diode arrays and dual-pathlength flow cells.

- All the light is allowed to pass through the sensing cell

- and *subsequently* the light is dispersed by means of a holographic grating and

- the dispersed light allowed to fall on an array of diodes.

Diode Array


Diode Array
Dispersion device
Entrance slit
Sample
Source

Diode Array

- The light (photons)generated electric charge carriers discharges the diode capacitor

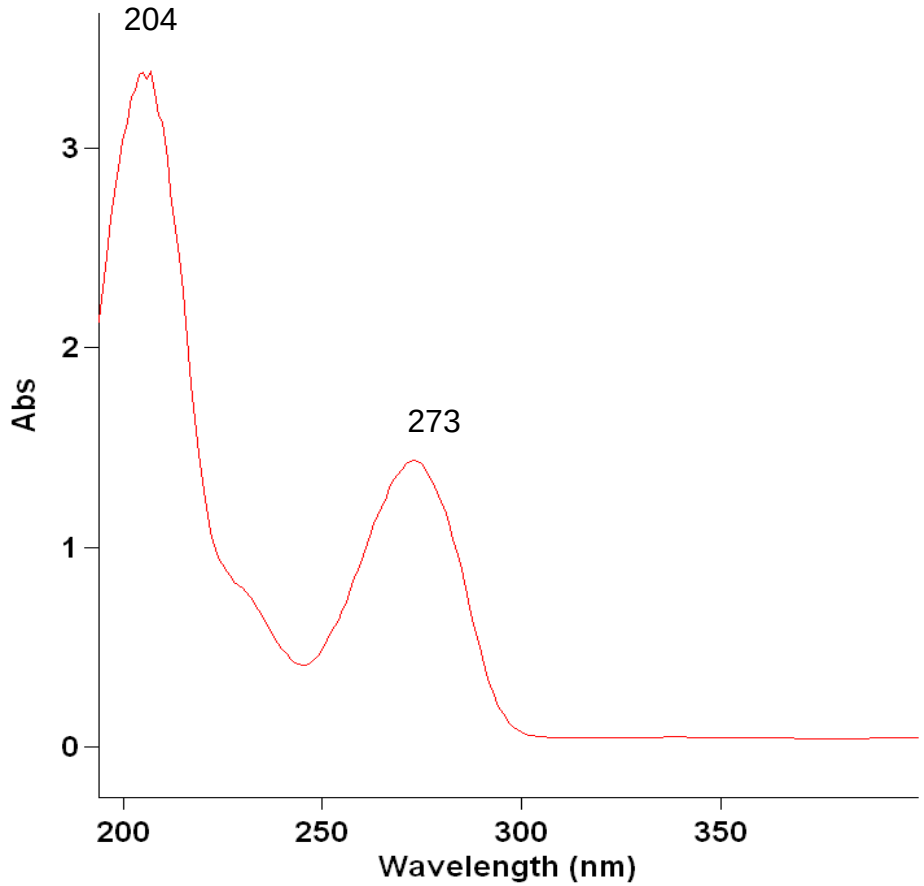
- The amount of charge needed to recharge capacitors is proportional to number of photons detected by each diode, which is proportional to light intensity.

HPLC Experiment

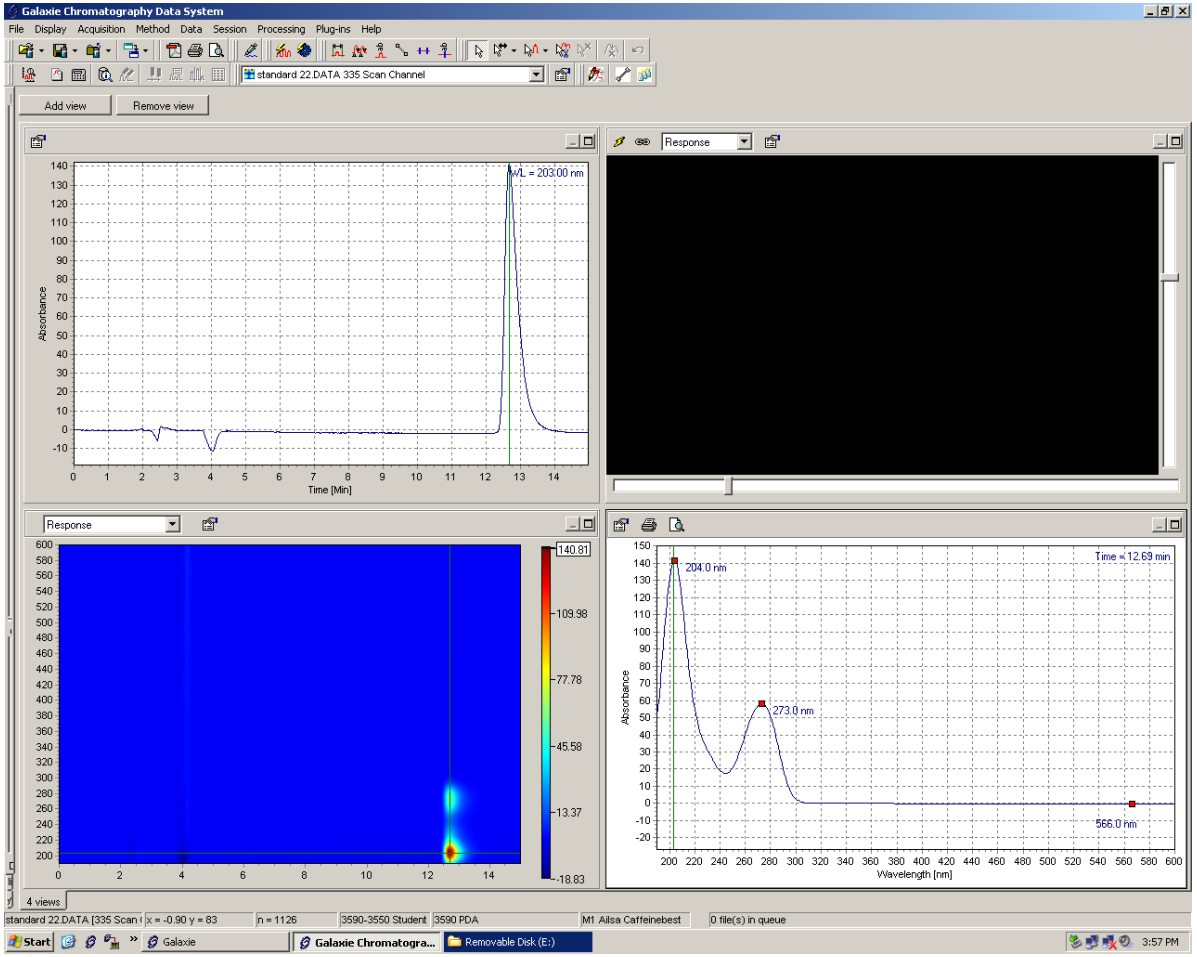
- Identification of unknown compounds by their retention time.

- Determination of concentration by use of
  1) external standard curve,
  2) internal standard or standard
  3) or standard addition.

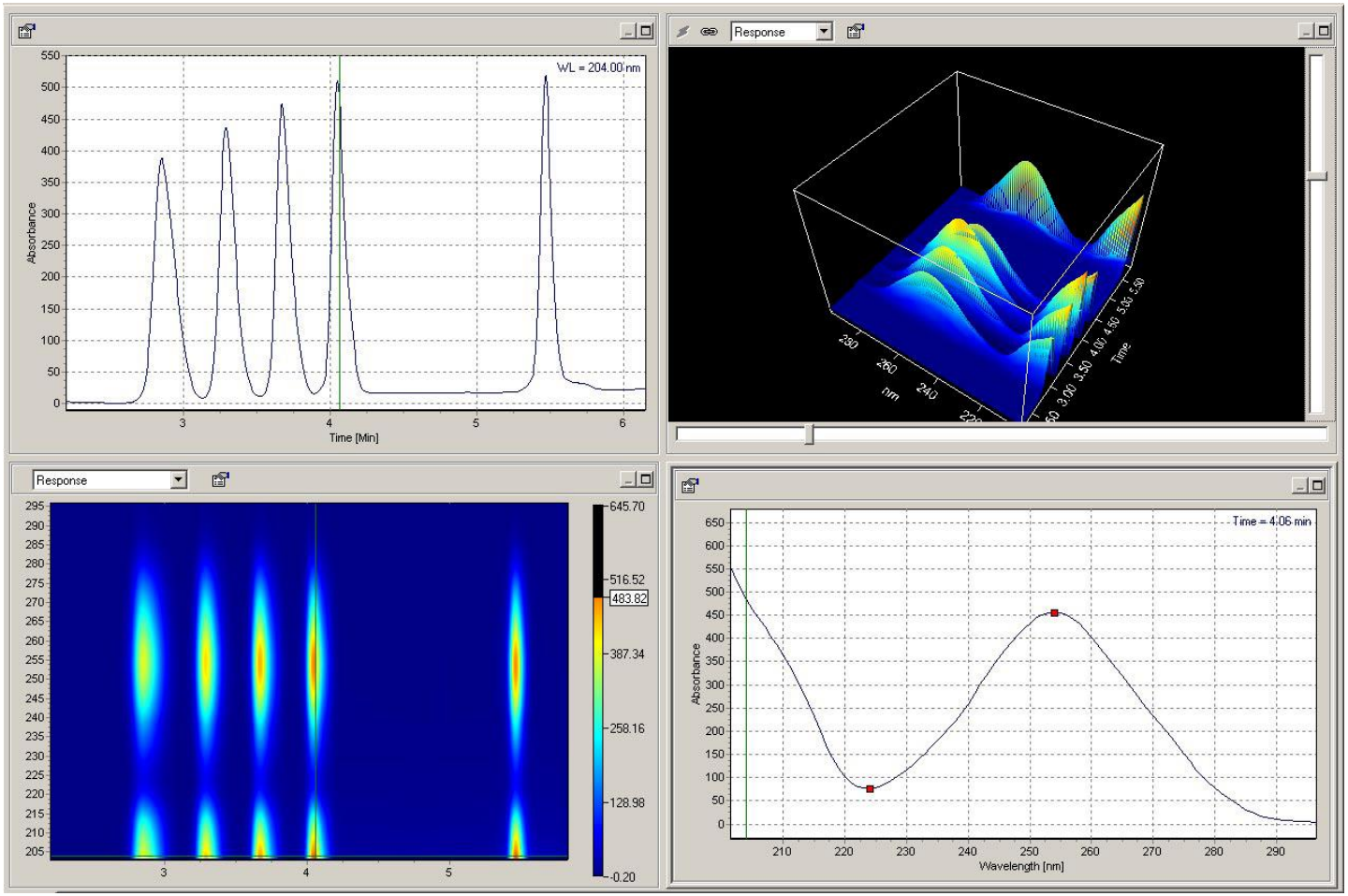
Cary 50 Scan Caffeine



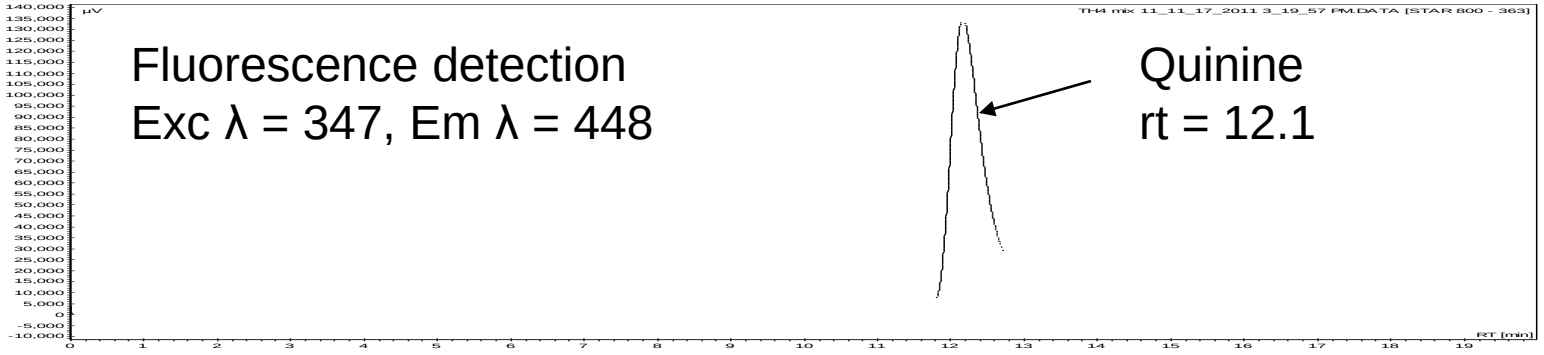
Diode Array Screen

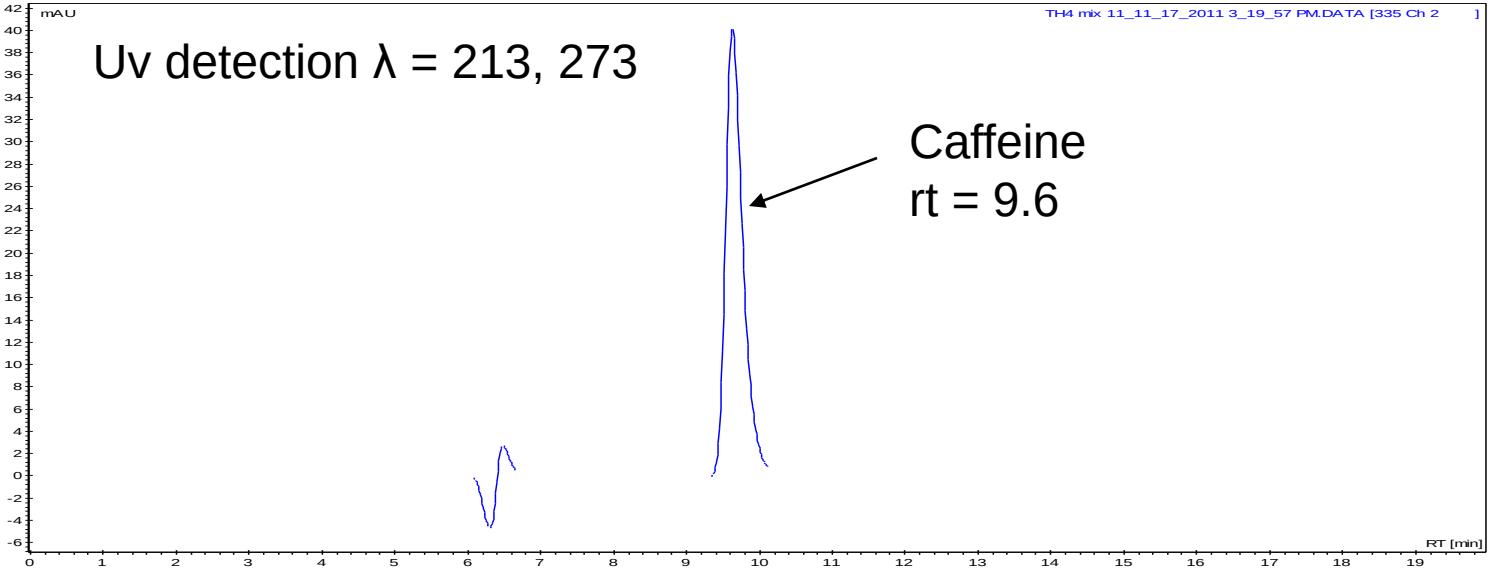


Four view display shows the chromatogram, 3D, absorbance and spectrum plots



Quinine and Caffeine



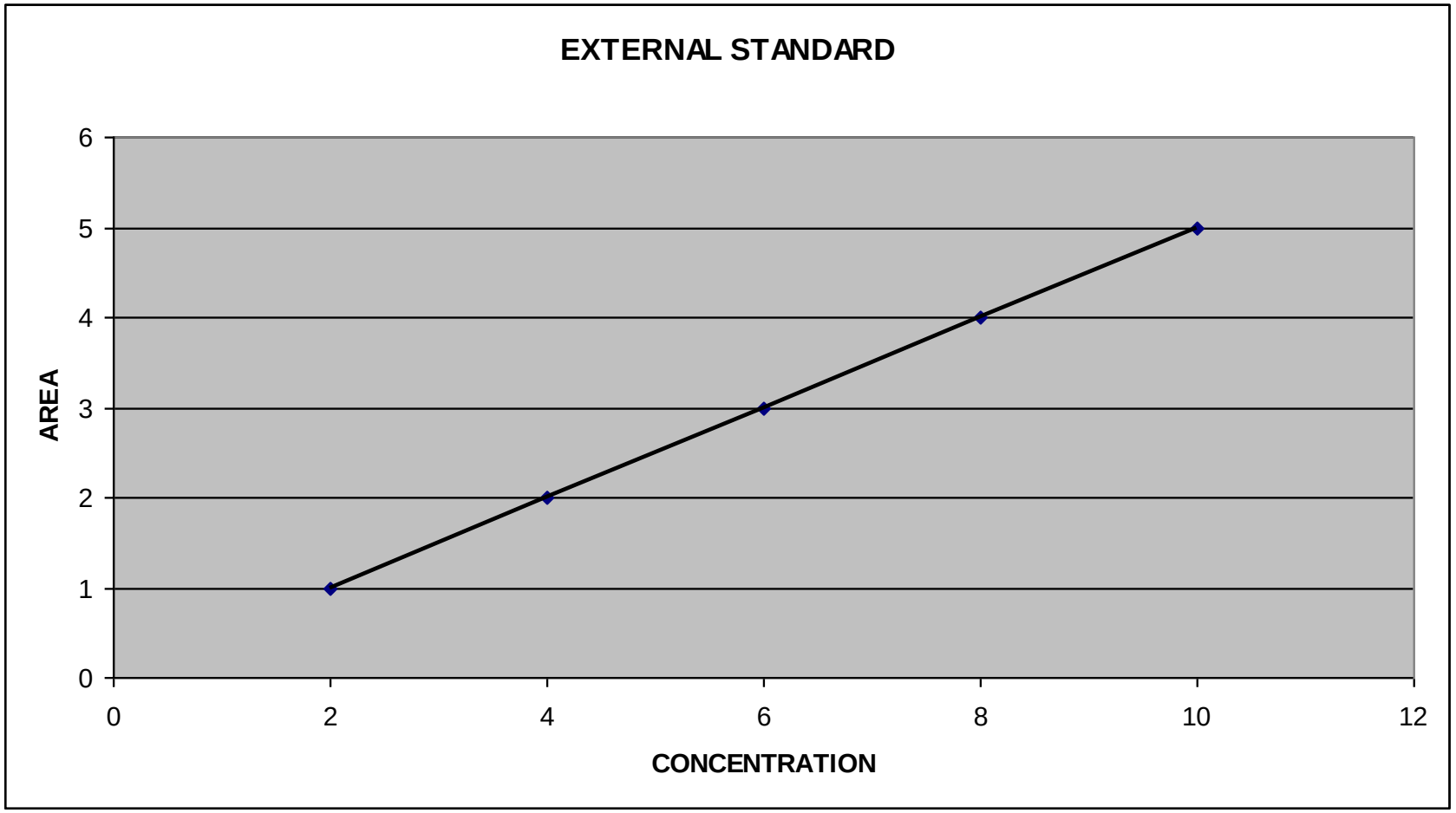


HPLC Experiment - Light Sources

- Fluorescence detector uses Xenon lamp which has wavelength region of 250-600

- The HPLC Diode Array uses two lamps a deuterium lamp with wavelengths from 160 to 380 and tungsten halogen lamp which covers wavelengths from 240 to 2500.

HPLC Experiment Concentration Determined by External Standard

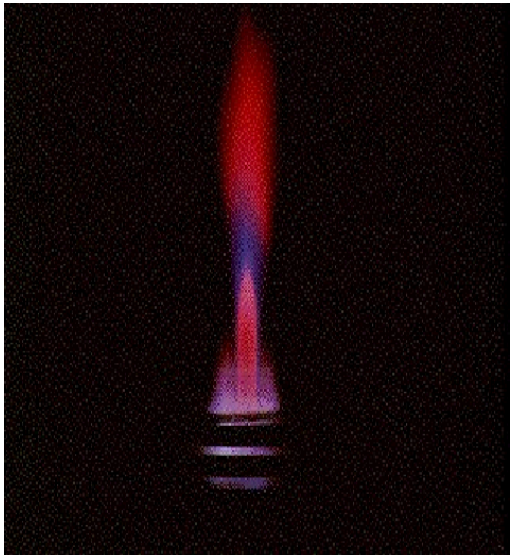


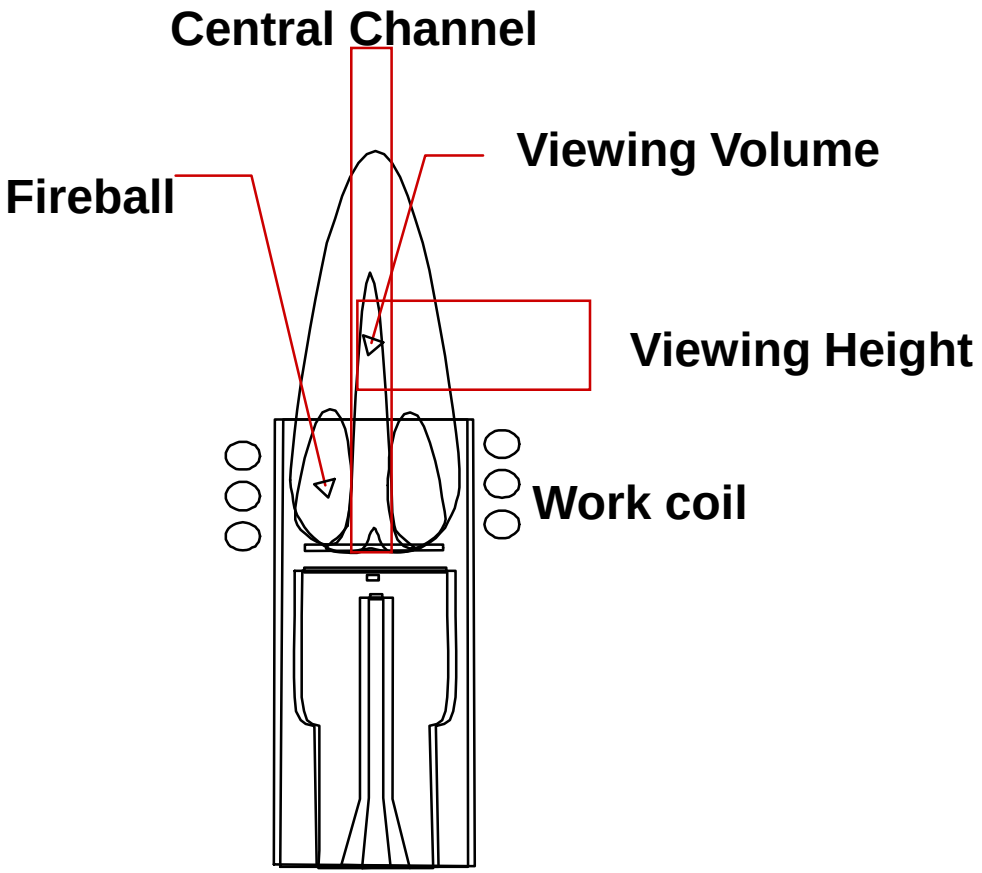
700-ES Series Simultaneous ICP's

ICP-OES Experiment

- Determine the mineral content of solid samples of:
  - Grains
  - Mining rock
  - Water
  - Feeds and foods
  - Surfaces

Radial Plasma

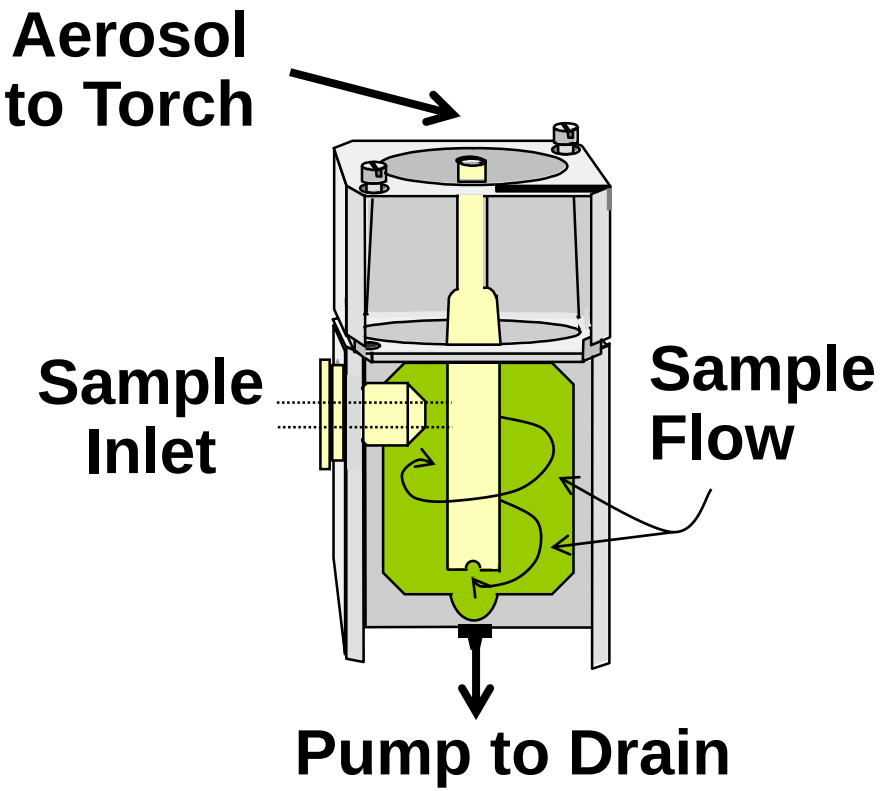




Spraychambers

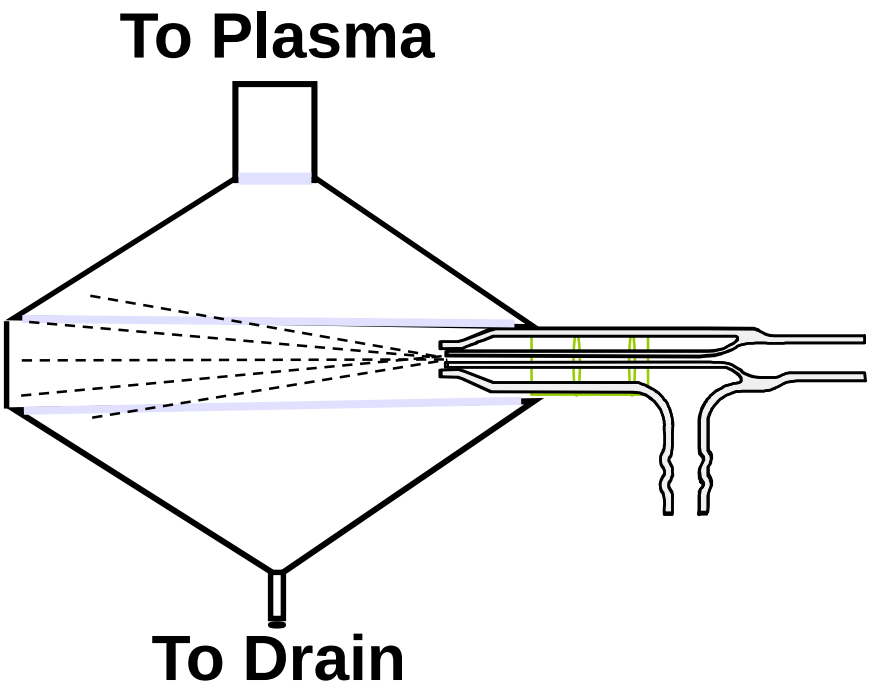
Sturman-Masters

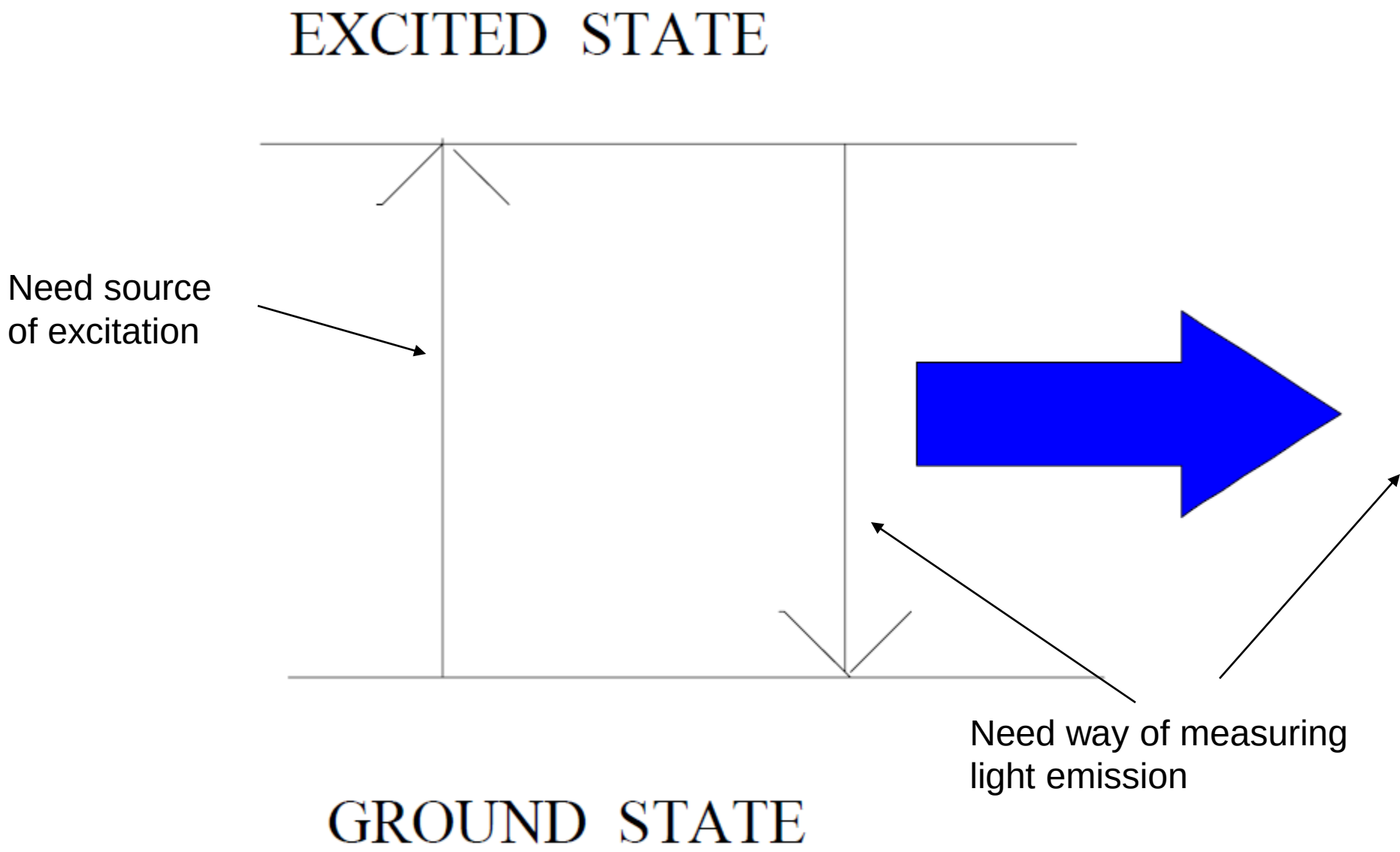
-double pass



Glass Cyclonic

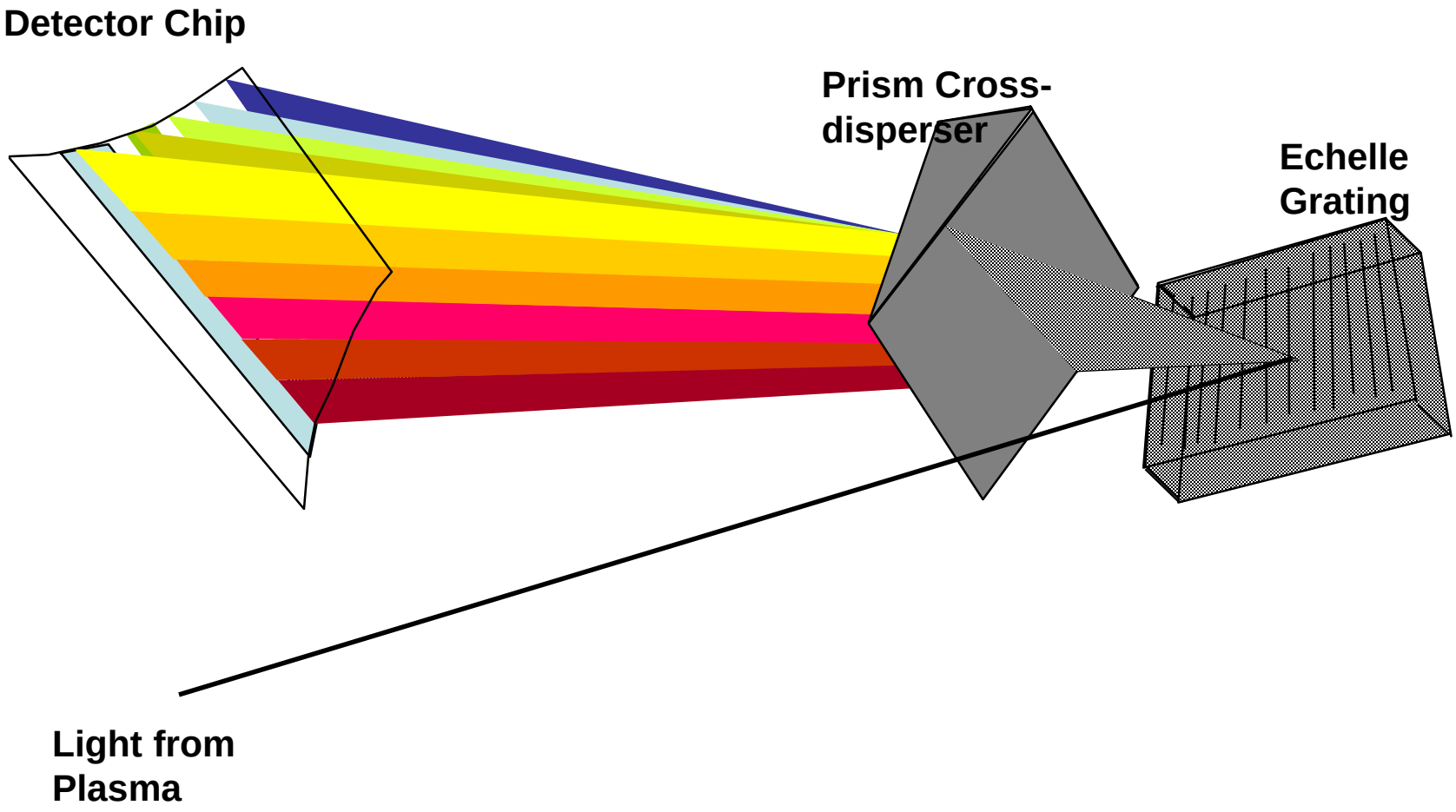
- smaller volume

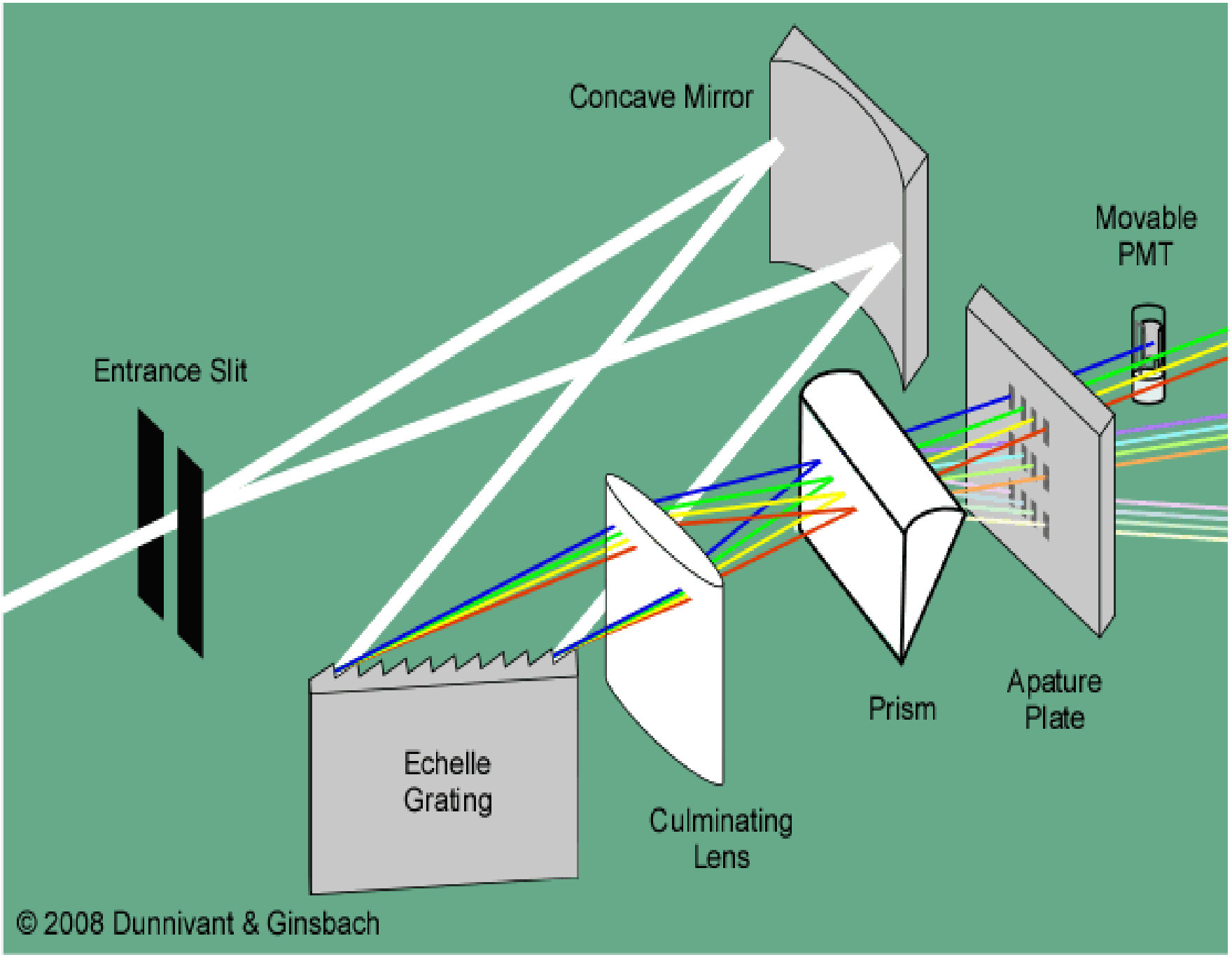




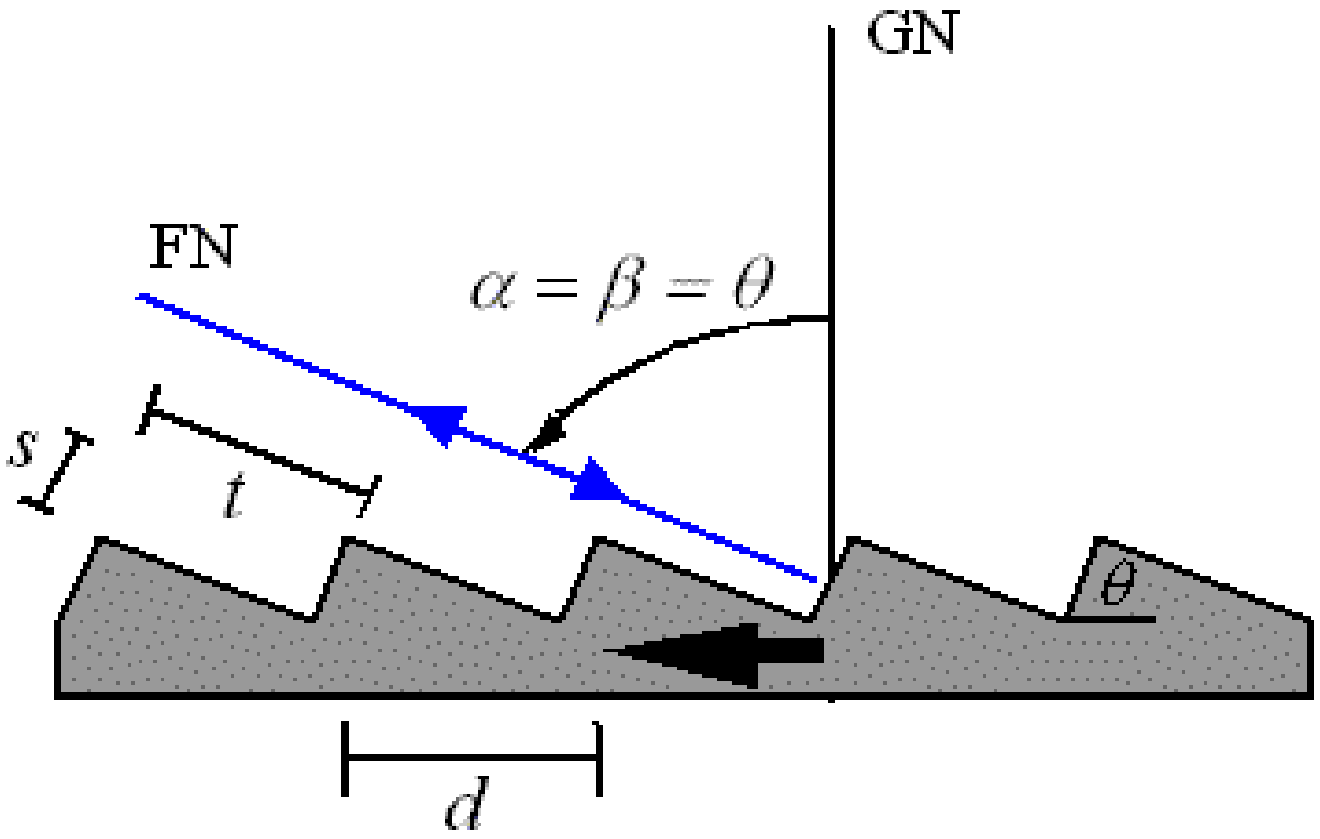
URE 1. EMISSION OF RADIATION UPON RELAXATION FROM AN EXCITED STATE.

Varian Simultaneous ICP Optics

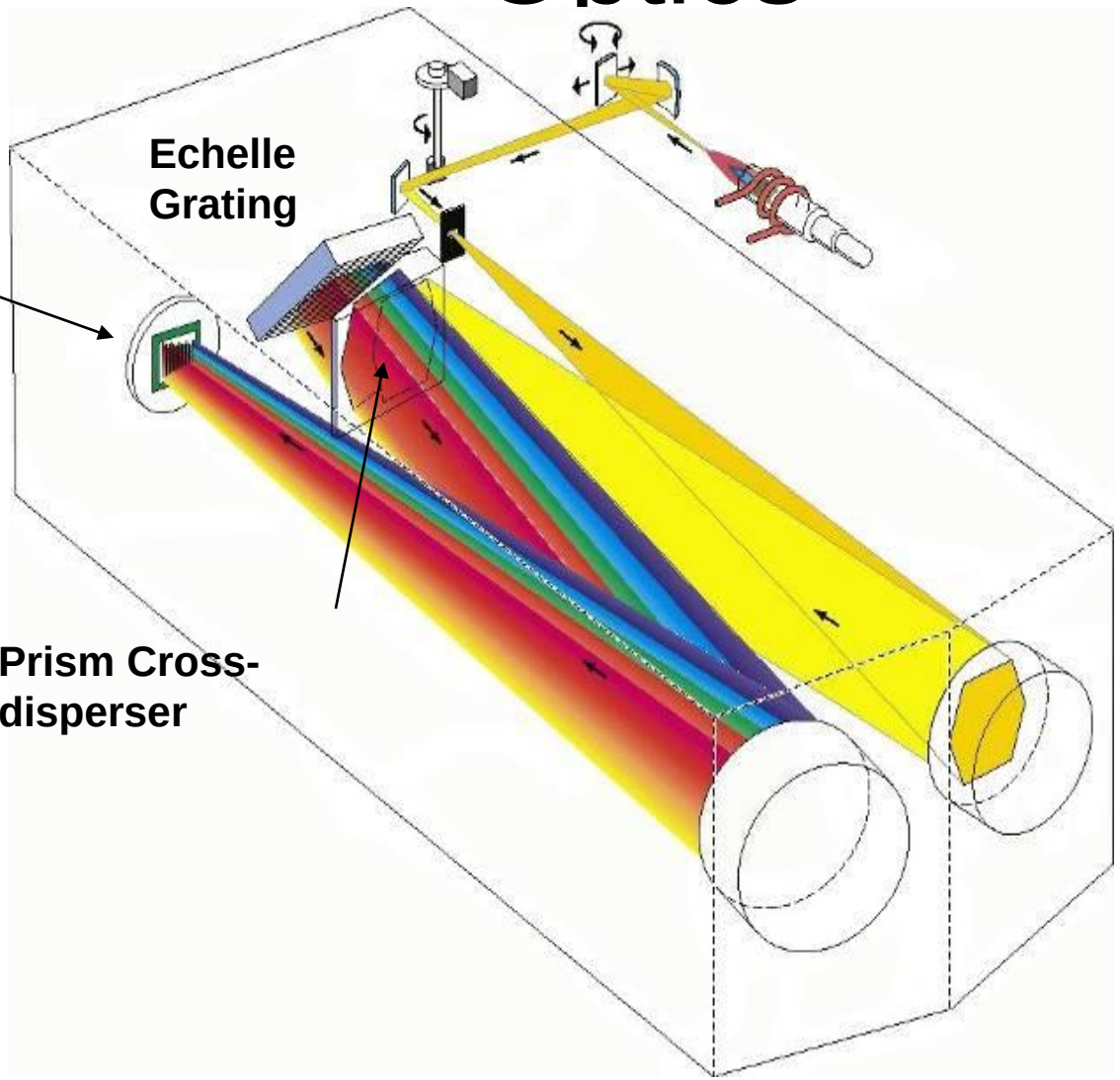



Concave Mirror
Movable PMT
Entrance Slit
Echelle Grating
Culminating Lens
Prism
Apature Plate
© 2008 Dunnivant & Ginsbach

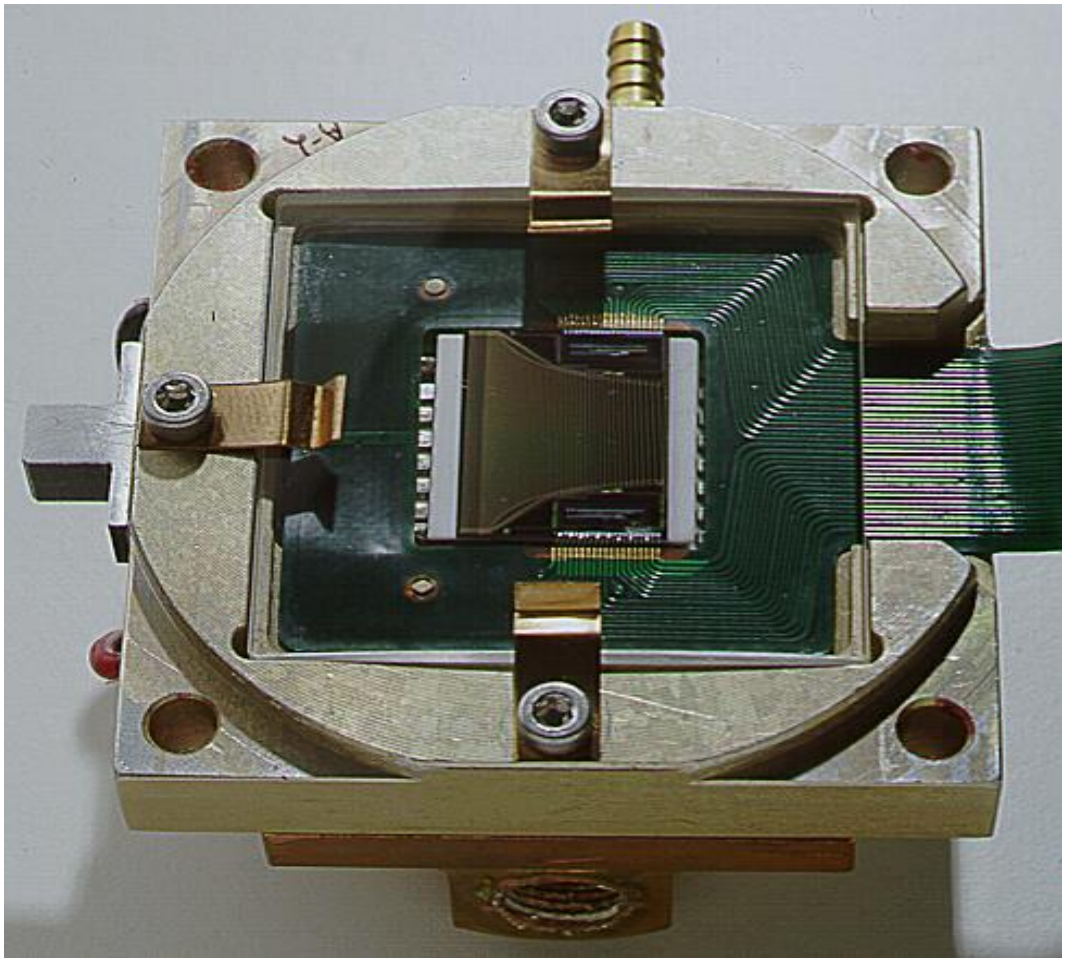
Echelle Grating



Varian Simultaneous ICP Optics



720-ES Series CCD Detector



VistaChip CCD detector

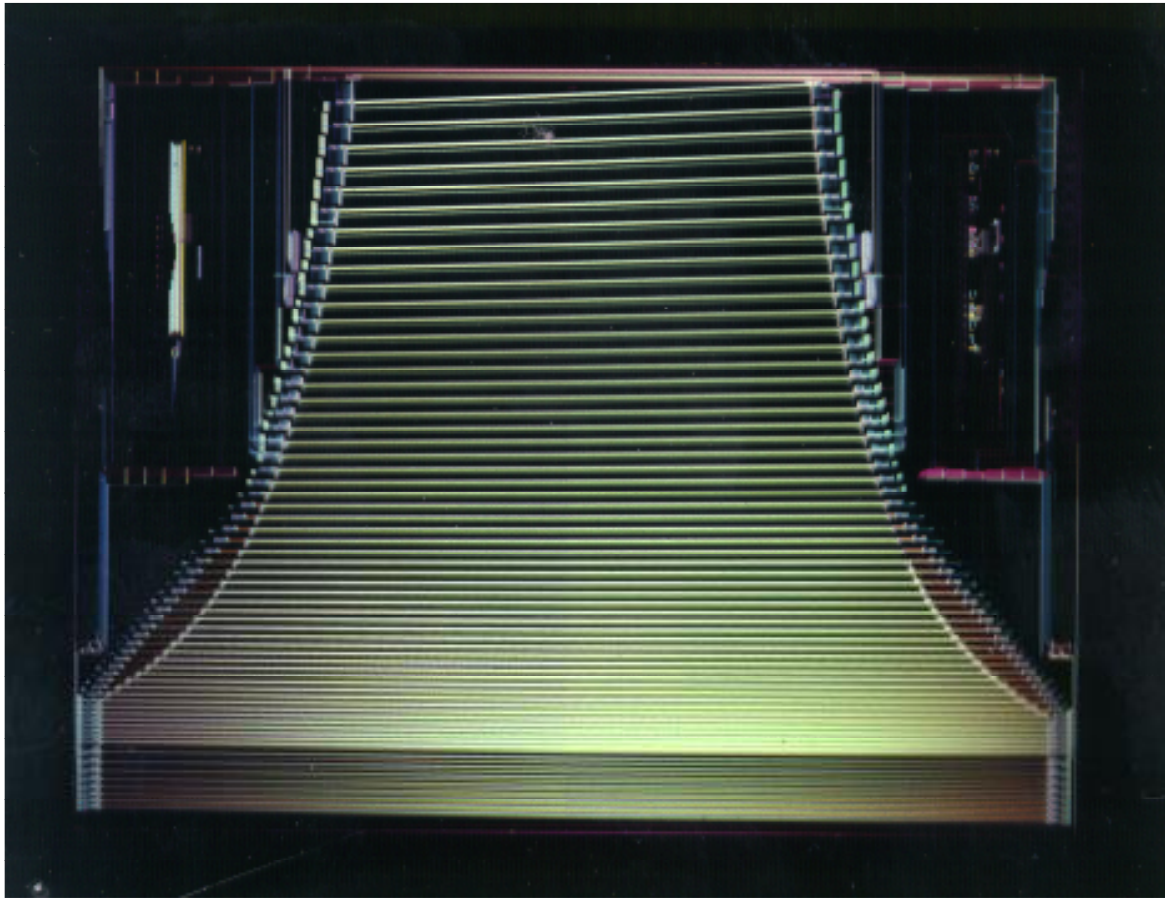
- The CCD (charge coupled device) 70 000 pixels, arranged to exactly match the two dimensional image from the echelle optics.
- continuous wavelength coverage from 167–785 nm.
- trace and major concentrations can be measured simultaneously,

Charged Coupled Device (CCD)

A charge coupled device (CCD) is an integrated circuit etched onto a silicon surface forming light sensitive elements called pixels. Photons incident on this surface generate charge that can be read by electronics and turned into a digital copy of the light patterns falling on the device

720-ES Series CCD Detector

Order 88



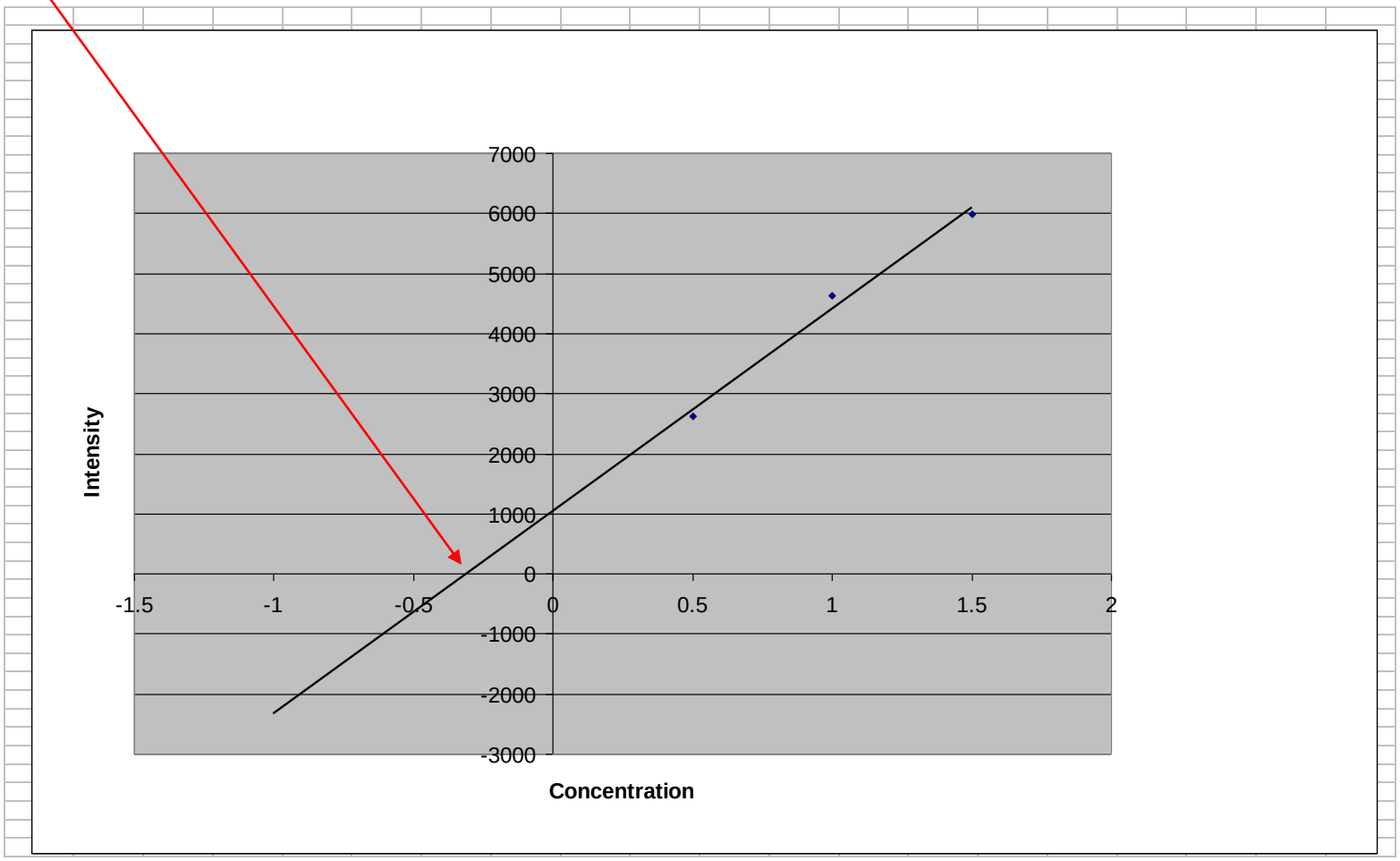
Order 19

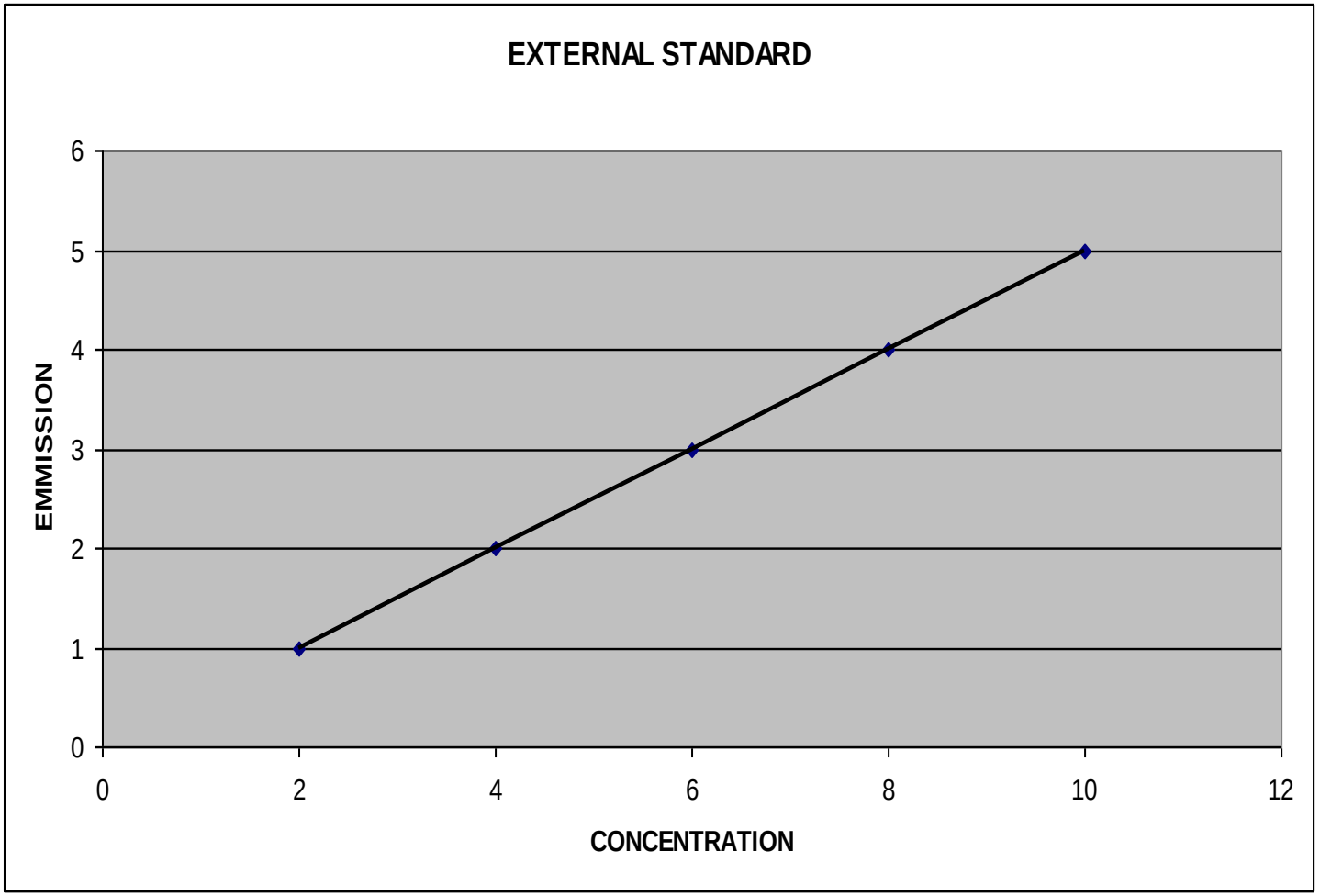
ICP Experiment

- Determination of mineral content in commercial products (product labelling, nutrient requirements, mining).
  - Dry ashing of sample
    - Burns off organic material leaving ash (minerals)
  - Acid digestion of sample
    - Dissolves minerals or can dissolve total sample including organic material (wet ashing)
- Concentration determined by external standard,standard addition, and internal standard.

Concentration of unknown

Standard Addition




EXTERNAL STANDARD
EMMISSION
0
1
2
3
4
5
6
0
2
4
6
8
10
12
CONCENTRATION